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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

APR 11, 1995

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: DICAMBA - Review of Subchronic Neurotoxicity Study (§82-7)

EPA ID NOs: MRID NO.: 432452-10
PC Code: 029801
Toxicology Chemical Code: 295
DP Barcode: D204480
Submission No.: S468139

FROM: Robert F. Fricke, Ph.D. Robert F. Fricke apr 5, 1995
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THRU: K. Clark Swentzel K. Clark Swentzel 4/6/95
Toxicology Branch II, Head, Section II
Health Effects Division (7509C)

and

Marcia van Gemert, Ph.D. Marcia van Gemert 4/11/95
Chief, Toxicology Branch II
Health Effects Division (7509C)

REGISTRANT: Sandoz Agro, Des Plaines, IL

CHEMICAL: Dicamba

ACTION REQUESTED: The Registrant has submitted a subchronic (§82-7) neurotoxicity studies in the rat with Dicamba for review.

1. **EXECUTIVE SUMMARY:** Crl:CD BR (Sprague-Dawley) rats (10 animals/sex/dose) were fed test diets containing 0 (basal diet), 3000, 6000, or 12000 ppm (0, 197.1, 401.4, 767.9, males; 0, 253.4, 472.0, or 1028.9 mg/kg/day, females) Dicamba for 13 weeks. Neurobehavioral evaluations, consisting of FOB, locomotor activity, and auditory startle response, were conducted at prestudy and during Weeks 4, 8 and 13.

No toxicologically significant differences were noted in either the mean body weights or food consumption of the treated animals.

Neurobehavioral evaluations at the 4-, 8-, and 13-week evaluations revealed abnormal FOB observations consisting of rigid body tone, slightly impaired righting reflex and impaired gait. At Week 13, the incidences of these findings were decreased. Rigid body tone was also noted during evaluation of the righting reflex and landing foot splay.

Based on the results of this study (rigid body tone, slightly impaired righting reflex and impaired gait), the LOEL of 12000 ppm was established in males (767.9 mg/kg/day) and females (1028.9 mg/kg/day); the NOEL was established at 6000 ppm in males (401.4 mg/kg/day) and females (472.0 mg/kg/day).

2. **CONCLUSIONS:** This study is classified as Core - Guideline and satisfies guideline requirements (§82-7) for a subchronic neurotoxicity screening battery in the rat.

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Reviewed by: Robert F. Fricke, Ph.D. Robert F. Fricke 5 Apr 95
Section II, Tox. Branch II (7509C)

Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 4/6/95
Section II, Tox. Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Neurotoxicity Study - Rat (§82-7)

EPA ID NOs: MRID NO.: 432452-10
Pesticide Chemical Code: 029801
Toxicology Chemical Code: 295
DP Barcode: D204480
Submission No.: S468139

TEST MATERIAL: Dicamba

SYNONYMS: 3,6-Dichloro-o-anisic acid

STUDY NOs: HWA 686-178

SPONSOR: Sandoz Agro, Inc., Des Plaines, IL

TESTING LAB: Hazelton Washington, Inc.
Vienna, Virginia 22182-1699

REPORT TITLE: Subchronic Neurotoxicity Study of Dietary Technical Dicamba in Rats

AUTHORS: D.J. Minnema

REPORT ISSUED: 15 February 1994

EXECUTIVE SUMMARY: Crl:CD BR (Sprague-Dawley) rats (10 animals/sex/dose) were fed test diets containing 0 (basal diet), 3000, 6000, or 12000 ppm (0, 197.1, 401.4, 767.9, males; 0, 253.4, 472.0, or 1028.9 mg/kg/day, females) Dicamba for 13 weeks. Neurobehavioral evaluations, consisting of FOB, locomotor activity, and auditory startle response, were conducted at prestudy and during Weeks 4, 8 and 13.

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reflex and impaired gait. At Week 13, the incidences of these findings were decreased. Rigid body tone was also noted during evaluation of the righting reflex and landing foot splay.

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This study is classified as Core - Guideline and satisfies guideline requirements (§82-7) for a subchronic neurotoxicity screening battery in the rat.

I. MATERIALS

A. Test Compound: Dicamba, technical; Description: beige flakes; Batch No: 52103810; Purity: 86.9%; Contaminants: Not given

B. Test Animals: Species: Rat; Strain: Sprague-Dawley, Crl:CD® BR; Age: 6 weeks; Weight at initiation (g): 253 to 297 (males), 155 to 187 (females); Source: Charles River Breeding Laboratories, Inc., Raleigh, NC; Housing: Individually in mesh-bottom cages; Feed: Purina Certified Rodent Chow #5002 *ad libitum*; Water: Tap water, *ad libitum*; Environment: Temperature, 65.6 to 80.6°F; Humidity, 32.8 to 89.1%; Light cycle (reversed) 12 hr/12 hr, light/dark; Air changes, not stated.

II. METHODS

A. Study Design: Animals were randomly assigned to control and treatment groups and fed diets at the indicated concentrations for at least 13 weeks (Table 1). Neurobehavioral assessment included evaluation in a Functional Observational Battery (FOB), automated auditory startle response and determination of locomotor activity. Neuropathological examination was performed at terminal sacrifice.

Table 1: Animal Assignment to Study Groups

Test Group	Dose (mg/kg)	Main Study	
		Male	Female
Control	0	10	10
Low	3000	10	10
Mid	6000	10	10
High	12000	0	10

B. Diet Preparation: For each dose level, concentrated premixes were prepared by blending sufficient amount of Dicamba with approximately 200 g of basal diet. The premixes were then mixed with basal diet to yield the intended concentrations. Diets were prepared weekly and stored at room temperature. Test diets were analyzed for homogeneity, stability, and achieved concentration.

C. Observations

1. Clinical signs: Animals were observed twice daily for signs of toxicity and mortality and once daily for clinical signs. Detailed clinical examinations were performed at each weighing interval.

2. Body weights: Animals were weighed on Day 0 (before dosing) and at weekly intervals, thereafter.

3. Food consumption: Food consumption was measured at weekly intervals.

4. Ophthalmological examinations: Ophthalmological examinations were performed at prestudy and at the termination of the study.

D. Neurobehavioral Assessment: Neurobehavioral assessment, consisting of the FOB and evaluation of motor activity, were performed at pre-study and during Weeks 4, 8, and 13 of treatment.

1. Functional Observational Battery: The following parameters were evaluated:

HOME CAGE/HAND-HELD OBSERVATIONS
- Appearance of fur
- Color of tears/deposits around eyes
- Convulsions/tremors
- Ease of handling/body tone
- Ease of removal from cage
- Excessive vocalizations
- Exophthalmus
- Lacrimation
- Palpebral closure
- Piloerection
- Respiration
- Salivation
- Writhing
- Other signs

PERFORMANCE MEASUREMENTS
- Hind/forelimb grip strengths
- Hindlimb landing foot splay
- Body temperature
- Tail flick latency

OPEN-FIELD OBSERVATIONS
- Arousal
- Circling
- Convulsions/tremors
- Gait
- Posture
- Abnormal/Stereotypic behavior
- Other signs

RESPONSE OBSERVATIONS
- Approach response
- Catalepsy withdrawal
- Olfactory response
- Pupillary reflex
- Air Righting reflex
- Touch response
- Automated auditory startle response

2. Locomotor activity: Motor activity was measured using automated photobeam activity recording devices. Animals were monitored individually over a 40 min session, consisting of eight 5-minute intervals.

F. Sacrifice and Pathology: Animals found dead or euthanized *in extremis* were examined grossly. At termination of the study, animals were weighed and euthanized with and an IP injection of pentobarbital. The first six main study animals were perfusion fixed *in situ* for neuropathological evaluation, the remaining animals were examined grossly. The tissues listed below were examined from control and high-dose animals. Tissues from

intermediate dose groups were collected and examined if necessary.

GROSS PATHOLOGY
- Carcass
- Cervical tissues and organs
- Cranial cavity
- External body surface
- All orifices
- External surface of the brain
- Nasal cavity and parinasal sinuses
- Thoracic, abdominal and pelvic cavities and viscera

NEUROPATHOLOGY
- Muscle (Anterior tibialis & gastrocnemius)
- Brain with brainstem (Medulla/pons cerebral cortex & cerebellar cortex)
- Cervical and lumbar dorsal root ganglia
- Gasserian ganglion
- Spinal cord (Cervical, lumbar & thoracic)
- Pituitary
- Eyes
- Peripheral nerves (Sciatic, seral & tibial)

G. Positive Controls: A positive control study (Study No.: HWA 0001-692, entitled "Neurotoxicity Study of Acrylamide (Positive Control) in Rats", dated 12 April 1994) submitted by the performing laboratory adequately validated the FOB, motor activity and neuropathology findings.

H. Statistical Evaluations: Parametric data were first evaluated using Levene's test to determine if the variances were homogeneous. Heterogeneous data were sequentially transformed ($\log_{10}x$, x^2, $\sqrt{x}$, $1/x$, arsine, or rank) and reevaluated using Levene's test. Homogeneous data were evaluated using analysis of variance (ANOVA) and, if significant differences were observed, pair-wise comparisons were carried out using Dunnett's multiple t-test. Continuous behavioral data were analyzed by factorial analysis of variance with repeated measures. Locomotor activity data were square-root transformed before statistical analysis. Dose effects and dose × time effects were detected using univariant analysis.

III. REGULATORY COMPLIANCES

A. Quality assurance was documented by signed and dated GLP and quality assurance statements.

B. A statement of "no confidentiality claims" was provided.

IV. RESULTS

A. Analytical Chemistry: Test diets were analyzed for stability, homogeneity and concentration. Samples taken from the top, middle and bottom of the low- and high-dose test diets indicated that the Dicamba was homogeneously distributed with relative standard deviations of 3.9 and 1.3%, respectively. The low- and high-dose test diets were stable for eight days at room temperature and 30 days when frozen. Analysis of the test

diets during the study showed that the achieved concentrations were 92.7 to 106%, 90.7 to 110% and 96.7 to 104% for the low-, mid-, and high-dose test diets.

B. Clinical Signs and Mortality: All animals survived to terminal sacrifice without the appearance of any treatment-related clinical signs.

C. Body Weight and Body Weight Gain: The mean body weights of high-dose males were significantly lower (6%) than controls at Week 4, and nonsignificantly lower at the weekly measurements, thereafter. The differences were considered minimal and not of toxicological significance.

D. Food Consumption and Achieved Compound Intake: No treatment-related differences in food consumption were observed during the study. Achieved compound intakes are summarized in Table 2.

Table 2: Achieved Compound Intake for Weeks 1 - 13 (Data summarized from study Table 5)

Sex	Achieved Dosage (mg/kg/day)		
	3000 ppm	6000 ppm	12000 ppm
Male	197.1	401.5	767.9
Female	253.4	472.0	1028.9

E. Ophthalmological Examinations: No treatment-related eye lesions were noted at the Week 14 examination.

F. Neurobehavioral Results

1. FOB Findings: Significant, treatment-related FOB findings were observed in high-dose males and females at the 4-, 8-, and 13-week evaluations (Attachment 1). Abnormal observations included rigid body tone, slightly impaired righting reflex and impaired gait. At Week 13, the incidences of these findings were decreased. Rigid body tone was also noted during evaluation of the righting reflex and landing foot splay.

2. Motor Activity: No treatment-related changes in locomotor activity were observed during the 4-, 8-, and 13-week evaluations.

G. Sacrifice and Pathology: No treatment-related gross or histopathological changes were observed.

V. DISCUSSION and CONCLUSIONS: Crl:CD BR (Sprague-Dawley) rats (10 animals/sex/dose) were fed test diets containing 0 (basal diet), 3000, 6000, or 12000 ppm (o, 197.1, 401.4, 767.9, males; 0, 253.4, 472.0, or 1028.9 mg/kg/day, females) for 13 weeks. Neurobehavioral evaluations, consisting of FOB, locomotor activity, and auditory startle response, were conducted at prestudy and during Weeks 4, 8 and 13.

No toxicologically significant differences were noted in either the mean body weights or food consumption of the treated animals.

Neurobehavioral evaluations, at the 4-, 8-, and 13-week evaluations, revealed abnormal, treatment-related FOB observations consisting of rigid body tone, slightly impaired righting reflex and impaired gait. At Week 13, the incidences of these findings were decreased. Rigid body tone was also noted during evaluation of the righting reflex and landing foot splay.

Based on the results of this study (rigid body tone, slightly impaired righting reflex and impaired gait), the LOEL of 12000 ppm was established in males (767.9 mg/kg/day) and females (1028.9 mg/kg/day); the NOEL was established at 6000 ppm in males (401.4 mg/kg/day) and females (472.0 mg/kg/day).

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HAZLETON WASHINGTON TABLE 6B STUDY NUMBER: 0686178
SUBCHRONIC NEUROTOXICITY STUDY OF DICAMBA IN RATS

FUNCTIONAL OBSERVATIONAL BATTERY SUMMARY TABLE FOR HOME CAGE: Handling/Body Tone

	SESSION 1 [0 Prior]		SESSION 2 [4 weeks]		SESSION 3 [8 weeks]		SESSION 4 [13 weeks]	
	M	F	M	F	M	F	M	F
GROUP 1 [HIGH]								
N	10	10	10	10	10	10	10	10
NORMAL	6	7	4	4	6	5	7	6
LIMP	0	0	0	0	0	0	0	0
ACTIVE	4	3	4	1	2	0	2	0
RIGID	0	0	2	5	2	5	1	4
DIFFICULT	0	0	0	0	0	0	0	0
GROUP 2 [CTRL]								
N	10	10	10	10	10	10	10	10
NORMAL	2	7	6	10	6	10	7	10
LIMP	0	0	0	0	0	0	0	0
ACTIVE	8	3	4	0	4	0	3	0
RIGID	0	0	0	0	0	0	0	0
DIFFICULT	0	0	0	0	0	0	0	0
GROUP 3 [MID]								
N	10	10	10	10	10	10	10	10
NORMAL	5	8	4	8	4	9	6	10
LIMP	0	0	0	0	0	0	0	0
ACTIVE	5	2	6	2	6	1	3	0
RIGID	0	0	0	0	0	0	0	0
DIFFICULT	0	0	0	0	0	0	1	0
GROUP 4 [LOW]								
N	10	10	10	10	10	10	10	10
NORMAL	7	8	9	8	8	10	8	10
LIMP	0	0	0	0	0	0	0	0
ACTIVE	3	2	1	1	2	0	2	0
RIGID	0	0	0	0	0	0	0	0
DIFFICULT	0	0	0	1	0	0	0	0

FUNCTIONAL OBSERVATIONAL BATTERY SUMMARY TABLE FOR OPEN FIELD: Gait

	SESSION 1 [0 Prior]		SESSION 2 [4 weeks]		SESSION 3 [8 weeks]		SESSION 4 [13 weeks]	
	M	F	M	F	M	F	M	F
GROUP 1 [HIGH]								
N	10	10	10	10	10	10	10	10
NORMAL	10	10	8	6	9	7	10	8
MILD IMPAIRED	0	0	2	4	1	3	0	2
MEDIUM IMPAIRED	0	0	0	0	0	0	0	0
VERY IMPAIRED	0	0	0	0	0	0	0	0
GROUP 2 [CTRL]								
N	10	10	10	10	10	10	10	10
NORMAL	10	10	10	10	10	10	10	10
MILD IMPAIRED	0	0	0	0	0	0	0	0
MEDIUM IMPAIRED	0	0	0	0	0	0	0	0
VERY IMPAIRED	0	0	0	0	0	0	0	0
GROUP 3 [MID]								
N	10	10	10	10	10	10	10	10
NORMAL	10	10	10	10	10	10	10	10
MILD IMPAIRED	0	0	0	0	0	0	0	0
MEDIUM IMPAIRED	0	0	0	0	0	0	0	0
VERY IMPAIRED	0	0	0	0	0	0	0	0
GROUP 4 [LOW]								
N	10	10	10	10	10	10	10	10
NORMAL	10	10	10	10	10	10	10	10
MILD IMPAIRED	0	0	0	0	0	0	0	0
MEDIUM IMPAIRED	0	0	0	0	0	0	0	0
VERY IMPAIRED	0	0	0	0	0	0	0	0

FUNCTIONAL OBSERVATIONAL BATTERY SUMMARY TABLE FOR RESPONSES: Righting Reflex

	SESSION 1 [0 Prior]		SESSION 2 [4 weeks]		SESSION 3 [8 weeks]		SESSION 4 [13 weeks]	
	M	F	M	F	M	F	M	F
GROUP 1 [HIGH]								
N	10	10	10	10	10	10	10	10
NORMAL	10	9	3	1	4	1	7	4
UNCOORDINATED	0	1	4	8	4	8	2	5
LANDS ON SIDE	0	0	2	1	2	1	1	1
LANDS ON BACK	0	0	1	0	0	0	0	0
GROUP 2 [CTRL]								
N	10	10	10	10	10	10	10	10
NORMAL	10	9	10	10	10	9	10	10
UNCOORDINATED	0	1	0	0	0	1	0	0
LANDS ON SIDE	0	0	0	0	0	0	0	0
LANDS ON BACK	0	0	0	0	0	0	0	0
GROUP 3 [MID]								
N	10	10	10	10	10	10	10	10
NORMAL	10	10	10	10	9	10	9	9
UNCOORDINATED	0	0	0	0	1	0	1	1
LANDS ON SIDE	0	0	0	0	0	0	0	0
LANDS ON BACK	0	0	0	0	0	0	0	0
GROUP 4 [LOW]								
N	10	10	10	10	10	10	10	10
NORMAL	10	9	8	10	8	10	9	9
UNCOORDINATED	0	1	2	0	2	0	1	1
LANDS ON SIDE	0	0	0	0	0	0	0	0
LANDS ON BACK	0	0	0	0	0	0	0	0

FUNCTIONAL OBSERVATIONAL BATTERY SUMMARY TABLE FOR RESPONSES: Other Signs

	SESSION 1 [0 Prior]		SESSION 2 [4 weeks]		SESSION 3 [8 weeks]		SESSION 4 [13 weeks]	
	M	F	M	F	M	F	M	F
GROUP 1 [HIGH]								
N	10	10	10	10	10	10	10	10
ABSENT	10	10	8	6	10	8	9	4
PRESENT	0	0	2[b]	4[b,c]	0	2[b,d]	1[b]	6[b]
GROUP 2 [CTRL]								
N	10	10	10	10	10	10	10	10
ABSENT	10	10	10	10	10	9	10	10
PRESENT	0	0	0	0	0	1[e]	0	0
GROUP 3 [MID]								
N	10	10	10	10	10	10	10	10
ABSENT	10	10	10	10	10	9	10	10
PRESENT	0	0	0	0	0	1[a]	0	0
GROUP 4 [LOW]								
N	10	10	10	10	10	10	10	10
ABSENT	9	10	10	10	10	10	10	10
PRESENT	1[a]	0	0	0	0	0	0	0

[a] Animal bit cotton swab during olfactory response test.
[b] Animal became rigid upon dropping during righting reflex.
[c] Animal experienced tremors when handled for righting reflex.
[d] Ears quivered upon landing of righting reflex.
[e] Ears quivered when handled.

FUNCTIONAL OBSERVATIONAL BATTERY SUMMARY TABLE FOR PERFORMANCE: Other Signs

	SESSION 1 [0 Prior]		SESSION 2 [4 weeks]		SESSION 3 [8 weeks]		SESSION 4 [13 weeks]	
	M	F	M	F	M	F	M	F
GROUP 1 [HIGH]								
N	10	10	10	10	10	10	10	10
ABSENT	10	10	9	6	10	8	9	6
PRESENT	0	0	1[a]	4[a]	0	0	1[a]	4[a, b]
GROUP 2 [CTRL]								
N	10	10	10	10	10	10	10	10
ABSENT	10	10	10	10	10	10	10	10
PRESENT	0	0	0	0	0	0	0	0
GROUP 3 [MID]								
N	10	10	10	10	10	10	10	10
ABSENT	10	10	10	10	10	10	10	10
PRESENT	0	0	0	0	0	0	0	0
GROUP 4 [LOW]								
N	10	10	10	10	10	10	10	10
ABSENT	9	10	10	10	10	10	10	10
PRESENT	1	0	0	0	0	0	0	0

[a] Animal became rigid upon dropping during landing foot splay.
[b] Animal fell on side during landing foot splay.